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(57) Abstract

Triglycerides with two or three different fatty acids chosen from the twelve essential fatty acids, oleic acid and other fatty acids containing 8 to 26 carbon atoms, useful in nutrition and in medicine.

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TRIGLYCERIDES

Field of Invention

The invention relates to triglycerides.

Background

The essential fatty acids (EFAs) consist of a series of twelve compounds illustrated in Table 1 below. Linoleic acid (LA) the parent compound of the n-6 series of EFAs, and alpha-linolenic acid (ALA) the parent compound of the n-3 series, are usually the main dietary EFAs. The parent compounds are metabolized by the sequence of reactions shown in Table 1. In quantitative terms, as judged by their levels in cell membranes and in other lipid fractions, dihomo-gamma-linolenic acid (DGLA) and arachidonic acid (AA) are the main EFA metabolites of the n-6 series, while eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the main metabolites of the n-3 series. DGLA, AA, EPA and DHA are important constituents of most of the lipids in the body. As well as being important in themselves they can also give rise to a wide range of oxygenated derivatives, the eicosanoids, including the prostaglandins, leukotrienes and other compounds.

The elongation reactions shown in Table 1, in which 2 carbon atoms are added to the chain, tend to be rapid, whereas the desaturation reactions in which an extra double bond is introduced tend to be very slow. Thus for example gamma-linolenic acid (GLA) is rapidly converted to DGLA while stearidonic acid is readily converted to 20:4n-3 and so these pairs of compounds are equivalent in dietary terms. However, DGLA is only slowly converted to AA. The reactions are not normally reversible nor, in man, are n-3 and n-6 series acids inter-convertible.

The table is as follows:-

TABLE 1

n-6		n-3
18:2 delta-9,12 (linoleic acid)		18:3 delta-9,12,15 (alpha-linolenic acid)
	delta-6 desaturase	
18:3 delta-6,9,12 (gamma-linolenic acid)	↓	18:4 delta-6,9,12,15 (stearidonic acid)
	elongation	
20:3 delta-8,11,14 (dihomo-gamma-linolenic acid)	↓	20:4 delta-8,11,14,17
	delta-5 desaturase	
20:4 delta-5,8,11,14 (arachidonic acid)	↓	20:5 delta-5,8,11,14,17 ('eicosapentaenoic acid')
	elongation	
22:4 delta-7,10,13,16 (adrenic acid)	↓	22:5 delta-7,10,13,16,19
	delta-4 desaturase	
22:5 delta-4,7,10,13,16	↓	22:6 delta-4,7,10,13,16,19 ('docosahexaenoic acid')

The acids, which in nature are of the all-cis configuration, are systematically named as derivatives of the corresponding octadecanoic, eicosanoic or docosanoic acids, e.g. delta-9, 12-octadecadienoic acid or delta-4,7,10,13,16, 19-docosahexaenoic acid, but numerical designations such as, correspondingly,

18:2 n-6 or 22:6 n-3 are convenient. Initials, for example, EPA for the 20:5 n-3 acid (eicosapentaenoic acid) or DHA for the 22:6 n-3 acid (docosahexaenoic acid), are also used but do not serve when n-3 and n-6 acids of the same chain length and degree of unsaturation exist as for example with the 22:5 acids. Trivial names in more or less common use in the n-6 series are as shown. Of the n-3 series only 18:3 n-3 has a commonly used trivial name, alpha-linolenic acid, though the name stearidonic acid is coming into use for the 18:4 n-3 acid and the names eicosapentaenoic acid and docosahexanenoic acid as such are also used.

It is becoming apparent that in many different disease states there are abnormalities of EFA biochemistry leading to abnormal EFA levels in various lipid fractions and in various tissues. These diseases include diseases of the heart and circulation such as hypertension and coronary and peripheral vascular disease, diseases of inflammation and immunity such as atopic disorders, osteoarthritis, rheumatoid arthritis, ulcerative colitis, Crohn's disease and various disorders going under the general classifications of inflammatory or auto-immune, neurological disorders such as Alzheimer's disease, Parkinson's disease and multiple sclerosis, disorders of the kidney, disorders of the skin, disorders of the gastrointestinal tract, disorders of metabolism of calcium and other minerals, disorders of bone and connective tissue, disorders of the reproductive and endocrine systems, psychiatric disorders including schizophrenia, and disorders of aging.

Different diseases have different problems in EFA metabolism and exhibit different abnormal patterns of EFAs and it is therefore desirable in some situations to give two or more of the EFAs simultaneously.

Furthermore, the EFAs are exceptionally susceptible to oxidation and so it may be appropriate to co-administer the EFAs with oleic acid (OA) which has potent properties as an antioxidant.

While the EFAs can be supplied in various forms and in various mixtures, it is in principle convenient in both nutrition and in medical treatment to be able to supply the fatty acids as predetermined, particular molecules. This is particularly true with respect to pharmaceuticals, where regulations and directives covering combination products are becoming steadily more restrictive. For example, in order to win government approval for a combination drug product containing compounds A, B and C, it is now no longer adequate to mix the three compounds together in formulation X, and then to compare X with placebo, P. Many governments now require proof of the value of each individual chemical entity, whether or not the whole point of a proposal is a synergistic action of different entities or a newly discovered simultaneous lack of more than one entity. Therefore at the very least clinical studies have to be set up comparing P with X, with A alone, with B alone and with C alone. Some governments might also require comparisons with A + B, A + C and B + C. Thus at least five and possibly eight groups would be required for testing with an enormous escalation of cost. In order to avoid this situation, it would be appropriate instead of having a mixture of A, B and C, to have a single molecule in which A, B and C are found together in the same chemical compound, Y, allowing direct and simple testing of Y against P with only two groups required. For this purpose triglycerides, which can contain three fatty acids, are proposed.

Triglyceride Structures

In triglycerides the different EFAs and oleic acid may be present in the same molecule, either randomly distributed among the 1, 2 and 3 positions or with a particular EFA being found specifically in one of the positions on the molecule. With each triglyceride one or two positions will be occupied by one fatty acid while the other one or two positions will be occupied by one or two other fatty acids.

In Preparing Triglycerides

The course of esterification may in principle be directed to favour a desired isomer, but if it is not, then the position of individual acid residues in the triglycerides produced from starting mixtures of two acids is one of the several possibilities:-

TABLE 2a

AAA AAB*

ABA ABB*

BAB

BBB

and for three different acids:-

TABLE 2b

AAA AAB* AAC*

ABA ABB* ABC*

ACA ACB* ACC*

BAB BAC*

BBB BBC*

BCB BCC*

CAC

CBC

CCC

with either isomer equally likely to be formed where C_2 is chiral, at least in chemical as opposed to enzymatic synthesis. What the two or three acids are, of course, depends on the choices made from the possible acids. What the preparations of the isomers are is calculable, for undirected synthesis.

A previous filing has described in detail a range of triglycerides for use in pharmaceuticals, nutritional supplements, foods and skin care preparations. In that filing it was suggested that the parent essential fatty acids, linoleic acid and alpha-linolenic acid had few biological actions themselves and so those fatty acids were excluded from the triglycerides described. The described triglycerides contained fatty acids which were divided into one of the following four categories:

a) GLA and DGLA.

b) AA, adrenic acid and the 22:5n-6 acid.

c) Stearidonic acid and the 20:4n-3 acid.

d) EPA, the 22:5n-3 acid and DHA.

Triglycerides were described which contained either two residues of a fatty acid from one group, and one residue selected from a different group, or which contained three different fatty acids selected from three different groups. These triglycerides optionally contained oleic acid as one of the fatty acid residues due to its potent antioxidant properties.

However, the concept that LA and ALA themselves have little value may have been overstated and it is probable that LA and ALA do have important actions in their own right. For example, linoleic acid appears to have specific actions on the skin (Prottey et al, Br J Dermatol 1977; 97:29-38) and recent unpublished studies (AC Buck) indicate that it may stimulate calcium absorption from the gut. ALA itself may have actions which result in a considerable reduction in the risk of myocardial infarction (M de Lorgeril et al, Lancet 1994; 343: 1454-9). It therefore seems that LA and ALA may have useful actions in their own right and that at the very least they do not have adverse effects and can act as useful carriers for the other fatty acids.

Furthermore, it may also be appropriate to co-administer the EFAs with a fatty acid other than oleic acid or the twelve essential fatty acids of the n-6 and n-3 series. Such fatty acids might include stearic acid, palmitic acid, lauric acid, myristic acid or any other fatty acid containing 8 to 26 carbon atoms, whether saturated, mono-unsaturated or polyunsaturated, and whether in the case of unsaturated fatty acids having its double bonds in either cis or trans configurations.

The Invention

In the light of the above the invention provides, as groups of isomers or singly, triglycerides containing:-

a) one residue of an acid selected from oleic acid and the following groups of acids:-

Group i) gamma-linolenic acid (GLA) and dihomo-gamma linolenic acid (DGLA)

Group ii) arachidonic acid (AA), adrenic acid, and the 22:5 n-6 acid

Group iii) stearidonic acid and the 20:4 n-3 acid

Group iv) eicosapentaenoic acid (EPA) the 22:5 n-3 acid, and docosahexaenoic acid (DHA),

one residue of an acid selected differently therefrom and one residue of an acid selected from linoleic acid, alpha-linolenic acid or any other fatty acid, other than oleic acid or the twelve n-6 and n-3 series essential fatty acids, containing 8 to 26 carbon atoms whether saturated, mono-unsaturated or polyunsaturated and whether in the case of unsaturated fatty acids having its double bonds in either cis or trans configurations; or

b) two residues of the same acid selected from oleic acid and the acids of groups i) to iv) above and one residue of an acid selected from alpha-linolenic acid or any other fatty acid, other than oleic acid or the twelve n-6 and n-3 series essential fatty acids, containing 8 to 26 carbon atoms whether saturated, mono-

unsaturated or polyunsaturated and whether in the case of unsaturated fatty acids having its double bonds in either cis or trans configurations; or

c) one residue of linoleic acid, one residue of alpha-linolenic acid and one residue of an acid selected from oleic acid and the acids of groups i) to iv) above; or

d) one residue of an acid selected from oleic acid and the acids of groups i) to iv) above, one residue of an acid selected from linoleic acid and alpha-linolenic acid and one residue of any other fatty acid, other than oleic acid or the twelve n-6 and n-3 series essential fatty acids, containing 8 to 26 carbon atoms whether saturated, mono-unsaturated or polyunsaturated and whether in the case of unsaturated fatty acids having its double bonds in either cis or trans configurations.

The groups of isomers so defined comprise mixtures of positional and/or optical isomers, which may be in the proportions arising from directed or undirected synthesis, or in proportions arising from treatment of as-synthesised mixtures to enhance the proportion of particular isomers or groups of isomers. Further, according to the method of synthesis and degree of enhancement if any, varying amounts of triglycerides other than those defined may also be present.

The selection of desired groups of isomers may also be tabulated as below, with arbitrary reference numbers for the triglycerides (TGs), or rather possible groups of triglyceride isomers represented. For example TG45 is the possible mono-linoleoyl - mono-alpha linolenoyl - mono- Group (iv) glycerides eg. the mono-linoleoyl) - mono - (alpha linolenoyl) - mono- (eicosapentanoyl) glyceride. The table is:-

TABLE 3

TG	LA	ALA	Oleic	Grp.1	Grp.2	Grp.3	Grp.4	Other FA
1	1	-	1	1	-	-	-	-
2	1	-	1	-	1	-	-	-
3	1	-	1	-	-	1	-	-
4	1	-	1	-	-	-	1	-
5	1	-	-	1	1	-	-	-
6	1	-	-	1	-	1	-	-
7	1	-	-	1	-	-	1	-
8	1	-	-	-	1	1	-	-
9	1	-	-	-	1	-	1	-
10	1	-	-	-	-	1	1	-
11	-	1	1	1	-	-	-	-
12	-	1	1	-	1	-	-	-
13	-	1	1	-	-	1	-	-
14	-	1	1	-	-	-	1	-
15	-	1	-	1	1	-	-	-
16	-	1	-	1	-	1	-	-
17	-	1	-	1	-	-	1	-
18	-	1	-	-	1	1	-	-
19	-	1	-	-	1	-	1	-
20	-	1	-	-	-	1	1	-
21	-	-	1	1	-	-	-	1
22	-	-	1	-	1	-	-	1

(Table 3 cont'd)

TG	LA	ALA	Oleic	Grp.1	Grp.2	Grp.3	Grp.4	Other FA
23	-	-	1	-	-	1	-	1
24	-	-	1	-	-	-	1	1
25	-	-	-	1	1	-	-	1
26	-	-	-	1	-	1	-	1
27	-	-	-	1	-	-	1	1
28	-	-	-	-	1	1	-	1
29	-	-	-	-	1	-	1	1
30	-	-	-	-	-	1	1	1
31	-	1	2	-	-	-	-	-
32	-	1	-	2	-	-	-	-
33	-	1	-	-	2	-	-	-
34	-	1	-	-	-	2	-	-
35	-	1	-	-	-	-	2	-
36	-	-	2	-	-	-	-	1
37	-	-	-	2	-	-	-	1
38	-	-	-	-	2	-	-	1
39	-	-	-	-	-	2	-	1
40	-	-	-	-	-	-	2	1
41	1	1	1	-	-	-	-	-
42	1	1	-	1	-	-	-	-
43	1	1	-	-	1	-	-	-
44	1	1	-	-	-	1	-	-

(Table 3 cont'd)

TG	LA	ALA	Oleic	Grp.1	Grp.2	Grp.3	Grp.4	Other FA
45	1	1	-	-	-	-	1	-
46	1	-	1	-	-	-	-	1
47	1	-	-	1	-	-	-	1
48	1	-	-	-	1	-	-	1
49	1	-	-	-	-	1	-	1
50	1	-	-	-	-	-	1	1
51	-	1	1	-	-	-	-	1
52	-	1	-	1	-	-	-	1
53	-	1	-	-	1	-	-	1
54	-	1	-	-	-	1	-	1
55	-	1	-	-	-	-	1	1

As well as in structural terms as above, the invention may be considered in terms of starting mixtures of acids, selected from oleic acid and the acids of Groups i) - iv) above, linoleic acid, alpha-linolenic acid and other C_{8-26} fatty acids, namely in molar terms (33% stands for one third, 66% stands for two thirds):-

i) 33% of an acid selected from oleic acid and the acids of Groups i), ii), iii) and iv), 33% of a different acid selected therefrom and 33% of linoleic acid, alpha linolenic acid, or any other C_{8-26} fatty acid other than oleic acid and the twelve n-6 and n-3 essential fatty acids; or

ii) 66% of an acid selected from oleic acid and the acids of Groups i), ii), iii) and iv) and 33% of an acid selected from alpha-linolenic acid or other C_{8-26} fatty acid other than oleic acid and the twelve n-6 and n-3 essential fatty acids; or

iii) 33% of linoleic acid; 33% of alpha-linolenic acid; and 33% of an acid selected from oleic acid and the acids of groups i), ii), iii) and iv); or

iv) 33% of an acid selected from oleic acid and the acids of Groups i), ii), iii) and iv), 33% of linoleic acid or alpha-linolenic acid and 33% of a C_{8-26} fatty acid other than oleic acid and the twelve n-6 and n-3 essential fatty acids.

Preferred starting mixtures, with arbitrary reference numbers for the triglycerides, or rather possible groups of triglyceride isomers (TGs), that they formally represent, are derived from Table 3, specifying numbers of residues, by reading 33 mole % (one third) for '1' and 66 mole % (two thirds) for '2'.

As the desire is to give mixed triglycerides of two or three acids, species AAA and BBB of Tables 2a and 2b are for example unwanted components of the synthesized mixture but the mixed species predominate and the as-synthesized mixtures are therefore valuable. Where the desire specifically is to give mixed triglycerides of three acids, such species do not predominate but are still present in a valuable proportion. In either case, desired species can be separated or part separated from others by chromatographic or other methods known in themselves.

Individual triglycerides either containing three different fatty acids or two fatty acids in a 2:1 ratio, may thus be manufactured by chemical or enzymatic means by methods known in themselves to those skilled in the art. If the method of synthesis or manufacture does not provide an adequate concentration of the desired triglyceride, then that triglyceride may be concentrated and purified by appropriate techniques as outlined later.

As far as we are aware, all the groups of triglyceride isomers defined as above consist of new triglycerides which do not appear in nature and have not previously been described. They may broadly be prepared as follows:

a) The individual fatty acids are purified from natural animal, vegetable or microbial sources or are chemically synthesized, there being methods known in themselves to those skilled in the art.

b) The individual fatty acids are then esterified with glycerol by chemical or enzymatic methods, there being again methods known in themselves to those skilled in the art. For example, the fatty acids and glycerol may be allowed to react together in the presence of one of a number of appropriate enzymes, or of p-toluene sulphonic acid hydrate.

c) If required, the specific triglycerides are further purified by appropriate methods, again known to those skilled in the art, in particular high pressure liquid chromatography or other appropriate forms of chromatography; low temperature crystallisation; or the use of solvents which differentially select triglycerides of particular composition.

In the product, desirably a specified particular triglyceride or group of triglycerides forms more than 10%, preferably more than 30% very preferably more than 70% and ideally more than 90% of the triglycerides present in any triglyceride mixture used for the preparation of pharmaceutical compositions, foods, or skin care products. The triglycerides may be made up into appropriate pharmaceuticals or foods so as to provide a daily dose of 1mg to 100g per day, preferably 10mg to 10g and very preferably 500mg to 4g. Alternatively in foods or skin care products the triglycerides may be incorporated in concentrations of 0.001 to 50%, preferably 0.05 to 20% and very preferably 0.1 to 5%.

The specified triglycerides have a wide variety of possible uses. They may be used as pharmaceuticals for the treatment or prevention of disease in which abnormalities of EFAs have been identified. They may be added to foods or be added to or used as nutritional supplements for those who require the particular EFAs for the treatment or prevention of disease. They may also be used in foods or pharmaceuticals for veterinary use. They may be used for skin care.

The triglycerides may be formulated in any way appropriate, as well known to those skilled in the art of preparing pharmaceuticals, skin care products

or foods. They may be administered orally, enterally, topically, parenterally, (subcutaneously, intramuscularly, intravenously or by any other route), rectally, vaginally or by any other appropriate route.

Synthetic Examples

The following are examples of synthesis of the triglycerides.

Several triglycerides have been prepared as examples of the range of triglycerides outlined in Table 3, as summarised in Table 4:-

TABLE 4

Code	Triglyceride	TG number (as in Table 3)
LGE	LA/GLA/EPA	7
GαLE	GLA/ALA/EPA	17
αLLG	ALA/LA/GLA	42

There are a large number of synthetic routes to triglycerides reported in the literature but two of these methods are of particular use. The first uses glycerol monoprotected with a 4-methoxybenzyl group as the starting point. At a later stage in the synthesis this group is removed using a boron reagent, but while this very efficiently removes the protecting group and minimises acyl group scrambling it also causes *cis-trans* isomerisation of the fatty acid double bonds. This fact coupled with the expense of the reagent severely limits the applicability of this route. The second main route starts with a base-catalysed epoxide ring opening of a glycidol by a fatty acid to yield a monoacyglycerol. This route does lead to a mixture of positional isomers but nevertheless has good potential for larger scale syntheses. Direct reaction between glycerol and a mixture of fatty acids mediated either by DCC/DMAP or p-toluenesulfonic acid, using a mixture of two different fatty acids

is a further useful synthetic pathway. Assuming that both fatty acids react at equal rates simple probability theory can be applied to predict the distribution of triglyceride products. For example when equal parts of 2 different fatty acids (A,B) are reacted with glycerol using p-toluenesulfonic acid catalysis. Four classes of triglyceride will be formed:

AAA	12.5%
AAB, ABA, BAA	37.5%
ABB, BAB, BBA	37.5%
BBB	12.5%

If the fatty acids are in a ratio of 2 parts A: 1 part B the theoretical preparations are:-

AAA	29.6%
AAB,ABA,BAA	44.4%
ABB,BAB,BBA	22.2%
BBB	3.7%

In actual measurements it should be noted that due to different extinction coefficients at 210nm (the monitoring wavelength used for the hplc analysis) the percentages measured by hplc are different to the theoretical values.

The same approach can be used to examine the distribution when 3 different fatty acids are used, when in reaction as above ten classes of triglyceride will be formed:

AAA	3.7%
BBB	3.7%
CCC	3.7%
AAB, ABA, BAA	11.1%
AAC, ACA, CAA	11.1%
BBA,BAB, ABB	11.1%
BBC, BCB, CBC	11.1%

CCA, CAC, ACC	11.1%
CCB, CBC, BCC	11.1%
ABC, ACB, BAC, BCA, CAB, CBA	22.2%

The invention is illustrated by the following preparative scheme, in which the following abbreviations occur:-

DCC	=	dicyclohexylcarbodiimide
DMAP	=	4-N,N-dimethylaminopyridine
LA	=	linoleic acid (*cis, cis* - 9, 12 - octadecadienoic acid)
ALA	=	alpha-linolenic acid (*cis, cis, cis* - 9, 12, 15 -octadecatrienoic acid)
GLA	=	γ-linolenic acid (*cis,cis,cis* - 6,9,12-octadecatrienoic acid)
EPA	=	*cis,cis,cis,cis,cis*-5,8,11,14,17-eicosapentaenoic acid
DHA	=	*cis,cis,cis,cis,cis,cis*, - 4,7,10,13,16,19-docosahexaenoic acid

The synthetic route for the preparation of the triglycerides LGE, GαLE and αLLG is as follows:-

Step (i) A mixture of GLA (95%, 2.0g, 7.2mmol), glycidol (560mg, 7.5mmol) and tri n-butylamine (40μl, 0.16mmol) was heated at 85°C under nitrogen for 5h. The reaction was then cooled and purified by flash chromatography (5% methanol / methylene chloride) to yield the product monoacylglycerol as a colourless oil. (Yield = 1g, 38%).

Step (ii) A solution of DCC (340mg, 1.63mmol) and DMAP (200mg, 1.63mmol) in methylene chloride (5 ml) was added to a solution of the monoacylglycerol from (i) (500mg, 1.42mmol) and LA (380mg, 1.35mmol) in methylene chloride (15 ml) at room temperature under nitrogen. After 3h the reaction mixture was diluted with hexane (25 ml), filtered, concentrated and

purified by flash chromatography (2% methanol / methylene chloride) to yield an isomeric mixture of diacylglycerols as a colourless oil. (Yield = 500mg, 58%.)

Step (iii) A solution of DCC (340mg, 1.63mmol) and DMAP (200mg, 1.63mmol) in methylene chloride (5ml) was added to a solution of the monoacylglycerol from (i) (500mg, 1.42mmol) and ALA (380mg, 1.35mmol) in methylene chloride (15 ml) at room temperature under nitrogen. After 3h the reaction mixture was diluted with hexane (25 ml), filtered, concentrated and purified by flash chromatography (2% methanol / methylene chloride) to yield an isomeric mixture of diacylglycerols as a colourless oil. (Yield = 500mg, 58%.)

Step (iv) A solution of DCC (110mg, 0.53mmol) and DMAP (65mg, 0.53mmol) in methylene chloride (5 ml) was added to a solution of the diacylglycerol (prepared as in (ii)) (250mg, 0.41mmol) and EPA (95%, 145mg, 0.47mmol) in methylene chloride (10ml) and the mixture was stirred at room temperature under nitrogen. After 2h the reaction mixture was diluted with hexane (50 ml), filtered, concentrated and purified by flash chromatography (5% ethyl acetate / hexane) to yield the pure triglyceride LGE as a colourless oil. (Yield = 300mg, 82%)

Step (v) A solution of DCC (110mg, 0.53mmol) and DMAP (65mg, 0.53mmol) in methylene chloride (5 ml) was added to a solution of the diacylglycerol (prepared as in (ii)) (250mg, 0.41mmol) and ALA (130mg, 0.47mmol) in methylene chloride (10 ml) and the mixture was stirred at room temperature under nitrogen. After 2h the reaction mixture was diluted with hexane (50 ml), filtered, concentrated and purified by flash chromatography (5% ethyl acetate / hexane) to yield the pure triglyceride αLLG as a colourless oil. (Yield 290mg, 79%).

Step (vi) A solution of DCC (220mg, 1.07mmol) and DMAP (130mg, 1.07mmol) in methylene chloride (10 ml) was added to a solution of the diacylglycerol (prepared as in (iii)) (500mg, 0.82mmol) and EPA (95%, 285mg, 0.94mmol) in methylene chloride (20 ml) and the mixture was stirred at room

temperature under nitrogen. After 2h the reaction mixture was diluted with hexane (50 ml), filtered, concentrated and purified by flash chromatography (5% ethyl acetate / hexane) to yield the pure triglyceride GαLE as a colourless oil. (Yield = 640mg, 87%).

The products of the above examples have been subject to hplc triglyceride analysis and gc fatty acid analysis using the following methods.

HPLC method for triglycerides

Column:	Techsphere 50DS 25cm x 4.6mm i.d., PP 55733
Solvent:	acetonitrile (65%): isopropanol (35%)
Flow:	1.5 ml/min
Detection:	210 nm

Gc method for fatty acid methyl esters

Column:	Supelcowax^{TM} 10 fused silica capillary column 30m x 0.53mm i.d. 1.00μm film thickness
Conditions:	Initial temperature 165°C (5 min) Level 1 gradient 2°C /min to 210°C Final temperature 210°C (15 min)

Summary of HPLC analyses

Triglyceride	*Retention time*	*% purity*
ALA / LA / GLA	9.33 min.	94.9
GLA / ALA / EPA	6.42 min.	85.3
LA / GLA / EPA	8.05 min.	88.9

Summary of gc analyses

ALA / LA / GLA

LA methyl ester	35.45%
GLA methyl ester	32.4%
ALA methyl ester	32.15%

GLA / ALA / EPA

GLA methyl ester	32.13%
ALA methyl ester	33.12%
EPA methyl ester	33.04%

LA / GLA / EPA

LA methyl ester	33.95%
GLA methyl ester	31.71%
EPA methyl ester	33.16%

Use Examples

The following are examples of modes of use of the triglycerides.

1. Any one or any mixture of the triglycerides specified in Table 3 made up in soft or hard gelatin capsules of any size between 100mg and 1g and administered to provide a daily dose of between 100mg and 10g.
2. Any one or any mixture of the specified triglycerides microencapsulated in gelatin or agar or any other appropriate material, or incorporated into any appropriate material to form a powder which can be taken orally, added to foods, tabletted, encapsulated, packed in sachets or any other appropriate form.
3. Any one or more of the specified triglycerides made up in a whip, liquid, cream or other appropriate form for oral administration.
4. Any one or more of the specified triglycerides made into a cream, ointment or other topical preparation at a concentration ranging from 0.1 to 30%.
5. Any one or more of the specified triglycerides made up into an emulsion suitable for parenteral administration.
6. Any one or more of the specified triglycerides added to any appropriate food material such as a spread, drink, candy, cereal, infant food or bakery product.

CLAIMS

1. As groups of isomers or singly, triglycerides containing:-

a) one residue of an acid selected from oleic acid and the following groups of acids:-

Group i) gamma-linolenic acid (GLA) and dihomo-gamma linolenic acid (DGLA)

Group ii) arachidonic acid (AA), adrenic acid, and the 22:5 n-6 acid

Group iii) stearidonic acid and the 20:4 n-3 acid

Group iv) eicosapentaenoic acid (EPA) the 22:5 n-3 acid, and docosahexaenoic acid (DHA),

one residue of an acid selected differently therefrom and one residue of an acid selected from linoleic acid, alpha-linolenic acid or any other fatty acid, other than oleic acid or the twelve n-6 and n-3 series essential fatty acids, containing 8 to 26 carbon atoms whether saturated, mono-unsaturated or polyunsaturated and whether in the case of unsaturated fatty acids having its double bonds in either cis or trans configurations; or

b) two residues of the same acid selected from oleic acid and the acids of groups i) to iv) above and one residue of an acid selected from alpha-linolenic acid or any other fatty acid, other than oleic acid or the twelve n-6 and n-3 series essential fatty acids, containing 8 to 26 carbon atoms whether saturated, mono-unsaturated or polyunsaturated and whether in the case of unsaturated fatty acids having its double bonds in either cis or trans configurations; or

c) one residue of linoleic acid, one residue of alpha-linolenic acid and one residue of an acid selected from oleic acid and the acids of groups i) to iv) above; or

d) one residue of an acid selected from oleic acid and the acids of groups i) to iv) above, one residue of an acid selected from linoleic acid and alpha-linolenic acid and one residue of any other fatty acid, other than oleic acid or the twelve n-6 and n-3 series essential fatty acids, containing 8 to 26 carbon atoms whether saturated, mono-unsaturated or polyunsaturated and whether in the case of unsaturated fatty acids having its double bonds in either cis or trans configurations.

2. Triglycerides according to claim 1, containing acid residues selected as set out in Table 3 herein under any one of the references TG1 to TG50.

3. Triglycerides according to claim 1 or 2, in the form of mixtures with other triglycerides, made by reaction of acid and glycerol starting materials in kinds and proportions corresponding to the selected residues.

4. Triglycerides according to claim 3, wherein the triglycerides according to claim 1 or 2 constitute more than 10 preferably more than 30 very preferably more than 70 and ideally more than 90 molar percent of the mixtures.

5. Triglycerides according to any preceding claim in the form of a pharmaceutical composition generally.

6. Triglycerides according to any of claims1 to 4 in the form of a nutritional supplement or food composition.

7. Triglycerides according to any of claims 1 to 4 in the form of a topical pharmaceutical, skin care or cosmetic composition.

8. Triglycerides according to claims 5, 6 or 7 wherein the composition is in a form to provide a daily dose of 1mg to 100g preferably 10mg to 10g and very preferably 500mg to 4g of triglyceride according to claim 1 or 2.

9. Triglycerides according to claims 5, 6 or 7 wherein the composition comprises 0.001 to 50% preferably 0.05 to 20% and very preferably 0.1 to 5% by weight of triglyceride according to claim 1 or 2.

INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/GB 96/00828

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07C69/587 A61K31/23 A23L1/30

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07C A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP,A,0 609 001 (SCOTIA HOLDINGS PLC) 3 August 1994 see page 6, line 26 - page 7, line 58 see page 18, line 1 - line 16 see page 18; claims	1,3-7
Y	WO,A,94 15464 (ABBOTT LABORATORIES) 21 July 1994 see page 11, paragraph 2 - page 13, paragraph 1 see page 23 - page 26; claims	1,3-7

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
17 July 1996	25.07.96
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Authorized officer Kinzinger, J

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 96/00828

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		AU-B-	5382194	04-08-94
		CA-A-	2114421	28-07-94
		CA-A-	2114422	28-07-94
		CN-A-	1105016	12-07-95
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WO-A-9415464	21-07-94	AU-B-	5995294	15-08-94
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